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Arabidopsis mutant analysis and gene regulation define a nonredundant role for glutamate dehydrogenase in nitrogen assimilation

(plant mutant/biochemical genetics/ammonia assimilation/gene expression)

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ABSTRACT **Glutamate dehydrogenase (GDH) is ubiquitous to all organisms, yet its role in higher plants remains enigmatic. To better understand the role of GDH in plant nitrogen metabolism, we have characterized an *Arabidopsis* mutant (*gdh1-1*) defective in one of two GDH gene products and have studied *GDH1* gene expression. *GDH1* mRNA accumulates to highest levels in dark-adapted or sucrose-starved plants, and light or sucrose treatment each repress *GDH1* mRNA accumulation. These results suggest that the *GDH1* gene product functions in the direction of glutamate catabolism under carbon-limiting conditions. Low levels of *GDH1* mRNA present in leaves of light-grown plants can be induced by exogenously supplied ammonia. Under such conditions of carbon and ammonia excess, GDH1 may function in the direction of glutamate biosynthesis. The *Arabidopsis gdh*-deficient mutant allele *gdh1-1* cosegregates with the *GDH1* gene and behaves as a recessive mutation. The *gdh1-1* mutant displays a conditional phenotype in that seedling growth is specifically retarded on media containing exogenously supplied inorganic nitrogen. These results suggest that GDH1 plays a nonredundant role in ammonia assimilation under conditions of inorganic nitrogen excess. This notion is further supported by the fact that the levels of mRNA for GDH1 and chloroplastic glutamine synthetase (GS2) are reciprocally regulated by light.**

Glutamate dehydrogenase (GDH; EC 1.4.1.2) serves as a link between carbon and nitrogen metabolism, as it is capable of assimilating ammonia into glutamate or deaminating glutamate into 2-oxoglutarate and ammonia. The relative importance of GDH versus nitrogen assimilatory enzymes such as glutamine synthetase (GS) has been deduced in microorganisms using mutants defective in either enzyme (1, 2). In plants, the importance of GDH in nitrogen assimilation has been under question since the discovery of the GS/GOGAT [glutamate synthase (glutamate 2-oxoglutarate aminotransferase)] cycle (3). Current opinion is divided as to whether GDH plays (*i*) a role in ammonia assimilation, particularly under high ammonia concentrations; (*ii*) a role in glutamate catabolism; or (*iii*) a redundant and dispensable role in nitrogen assimilation (4–7).

The proposed roles for GDH in plants have been based largely on *in vitro* studies that have uncovered two types of GDH enzymes, an NADPH-requiring GDH enzyme that is localized to chloroplasts and an NADH-requiring GDH found in the mitochondria (6, 8). The GDH enzymes from a variety of higher plants exhibit high K_m values for ammonia (>1 mM), which argues against a major role of GDH in primary nitrogen assimilation *in vivo* (9). Because high levels of photorespiratory ammonia are released in mitochondria, it has been proposed that mitochondrial NADH-GDH plays a major role in reassimilating photorespiratory ammonia (6). However, several pieces of data argue against this proposed role. Inhibitors of GS, such as phosphinothricin, specifically kill plants grown under photorespiratory growth conditions (4, 10, 11). Second, the characterization of photorespiratory mutants has supported a major role for GS/GOGAT in this process. Plant mutants deficient in chloroplast GS2 or ferredoxin-dependent GOGAT are chlorotic when grown under photorespiratory conditions (in air), yet they display a normal phenotype when grown under conditions that suppress photorespiration (high CO_2) (12–15). Together these data suggest that GDH plays a minor role, if any, in the reassimilation of photorespiratory ammonia. An alternate role has been proposed in which GDH functions in ammonia detoxification, because its activity is increased in plants exposed to high levels of ammonia (16). Finally, a catabolic function for GDH has been proposed to be important for remobilization of ammonia from glutamate during germination, senescence, and seed set (6, 7).

Despite decades of biochemical studies on plant GDH, the *in vivo* role of this enzyme in plant nitrogen metabolism remains equivocal. Because the mechanisms controlling the intra- and intercellular transport of inorganic nitrogen and organic nitrogen are presently unknown, the *in vivo* function of GDH can best be judged by characterizing the phenotype of plant mutants defective in GDH. A plant mutant deficient in GDH has been previously isolated from maize (17, 18). However, this maize GDH-deficient mutant cannot be used to assess the *in vivo* role of GDH in photorespiration, because C_4 plants display low or negligible photorespiratory rates. Here, we report the characterization of a plant *GDH* gene, analyze its regulation by light and/or metabolites, and characterize an *Arabidopsis* mutant (*gdh1-1*) deficient in one of two GDH gene products. This molecular–genetic dissection in *Arabidopsis* indicates that GDH plays a nonredundant role in plant nitrogen metabolism in a C_3 plant.

MATERIALS AND METHODS

Plant Lines and Growth Conditions. The Columbia ecotype of *Arabidopsis* was used in all experiments, unless otherwise noted. The Landsberg ecotype was used to determine a restriction fragment length polymorphism for *GDH1*. Recombinant inbred lines were obtained from the Ohio State University, *Arabidopsis* Stock Center (19). Plants were grown at 45 $mE \cdot m^{-2} \cdot sec^{-1}$ (E = einstein, 1 mol of photons) on a 16-h light/8-h dark cycle unless otherwise indicated. "Light-grown" and "dark-adapted" plants were grown initially under the normal day/night light regime and were subsequently transferred to continuous light or dark, respectively, for 48 h before

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Abbreviations: GDH, glutamate dehydrogenase; GS, glutamine synthetase; GOGAT, glutamate synthase (glutamate 2-oxoglutarate aminotransferase); MS, Murashige and Skoog.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. U53527).

harvest for RNA isolation. For RNA isolation, plants were grown on Murashige and Skoog (MS) salts (GIBCO/BRL, catalog no. 11117) under "semihydroponic" conditions, unless otherwise noted. For semihydroponics, seeds were sown on nylon nets (Tetko, Elmsford, NY, catalog no. 3–250/50) suspended on MS media containing 3% sucrose and 0.4% agar and grown for 16–18 days. Thereafter, the nylon net was lifted, and the plants were transferred to fresh MS medium containing the indicated supplementations. Ethyl methanesulfonate- and methylnitrosourea-mutagenized *Arabidopsis* seeds (Columbia ecotype) were obtained from R. Last (Cornell University). For mutant screening, M_2 mutagenized seeds were plated on MS media supplemented with 0.05% aspartate. The ammonia-free, nitrate-containing MS medium (Sigma, catalog no. M2909; used in Fig. 5) and the ammonia-free/nitrate-free MS media (Sigma catalog no. M0529; used in Fig. 7) were supplemented with the appropriate MS salts and vitamins, unless otherwise noted.

RNA and DNA Manipulations. RNA extraction (20), DNA extraction (21), and Northern and Southern blot analyses (22) were performed as described. The 16S ribosomal RNA cDNA probe (rRNA, provided by B. Scheres, University of Utrecht, Utrecht, the Netherlands), a PCR-generated α-tubulin exon 4 probe, spanning nucleotides 1209–1596 (a gift from C. Silflow, University of Minnesota), and a full-length cDNA for the chloroplastic form of GS2 (GLN2; ref. 23) were labeled by the random primed method. Probe labeling, prehybridization, hybridization, and detection were as indicated in the Genius System *User's Guide for Membrane Hybridization* (Boehringer Mannheim). The membranes were washed at either low stringency (1× SSC, 65°C) or high stringency (0.1× SSC, 65°C). Blots were exposed to x-ray film, and the signals were quantified using the National Institutes of Health IMAGE version 1.41 software and normalized to the internal control gene.

Characterization of GDH1 cDNA. The deduced *Arabidopsis* GDH1 protein in Fig. 1 was obtained from the complete sequence analysis of a cDNA identified in an expressed sequence tag (EST) library from *Arabidopsis* (EST clone 134D5T7) (24). The *GDH1* gene-specific probe was a 360-nt, single-stranded, digoxigenin-labeled DNA probe spanning amino acids 42–150 of the GDH protein (Fig. 1, underlined residues), generated by PCR (25).

Mapping the *gdh1-1* Mutation and the *GDH1* Gene. PCR and simple sequence length polymorphism markers for mapping the *gdh1-1* mutation were as described (26). The *GDH1* gene was mapped using recombinant inbred lines (19). A restriction fragment length polymorphism (27) for *GDH1* was identified between Columbia and Landsberg using the endonuclease *Hha*I. *Hha*I digests of DNA from 25 different recombinant inbred lines were used to define the parental *GDH1* gene (19). The segregation data were analyzed, and the *GDH1* gene was then mapped relative to 462 markers by C. Lister (John Innes Centre, Norwich, U.K.).

Native Gel GDH Assay. Leaves from 21-day-old *Arabidopsis* plants were ground in 20 μl of extraction buffer (28) and electrophoresed through a discontinuous gel system under nondenaturing conditions as described (29). GDH activity staining was performed using nitroblue tetrazolium, as described (30). Total protein was determined by the method of Bradford (31).

RESULTS

Deduced Primary Sequence of the *Arabidopsis* GDH1 Protein and Conserved Features. The deduced *Arabidopsis* GDH1 protein shown in Fig. 1 shares a high overall identity with the GDH proteins of archaeobacteria (41%; ref. 32), humans (31%; ref. 33), yeast (27%; ref. 34), *Chlorella* (27%; ref. 35), and *Escherichia coli* (27%; ref. 36). Comparative analysis reveals that the encoded *Arabidopsis* GDH1 protein contains the hallmark residues conserved amongst all GDH proteins (Fig.

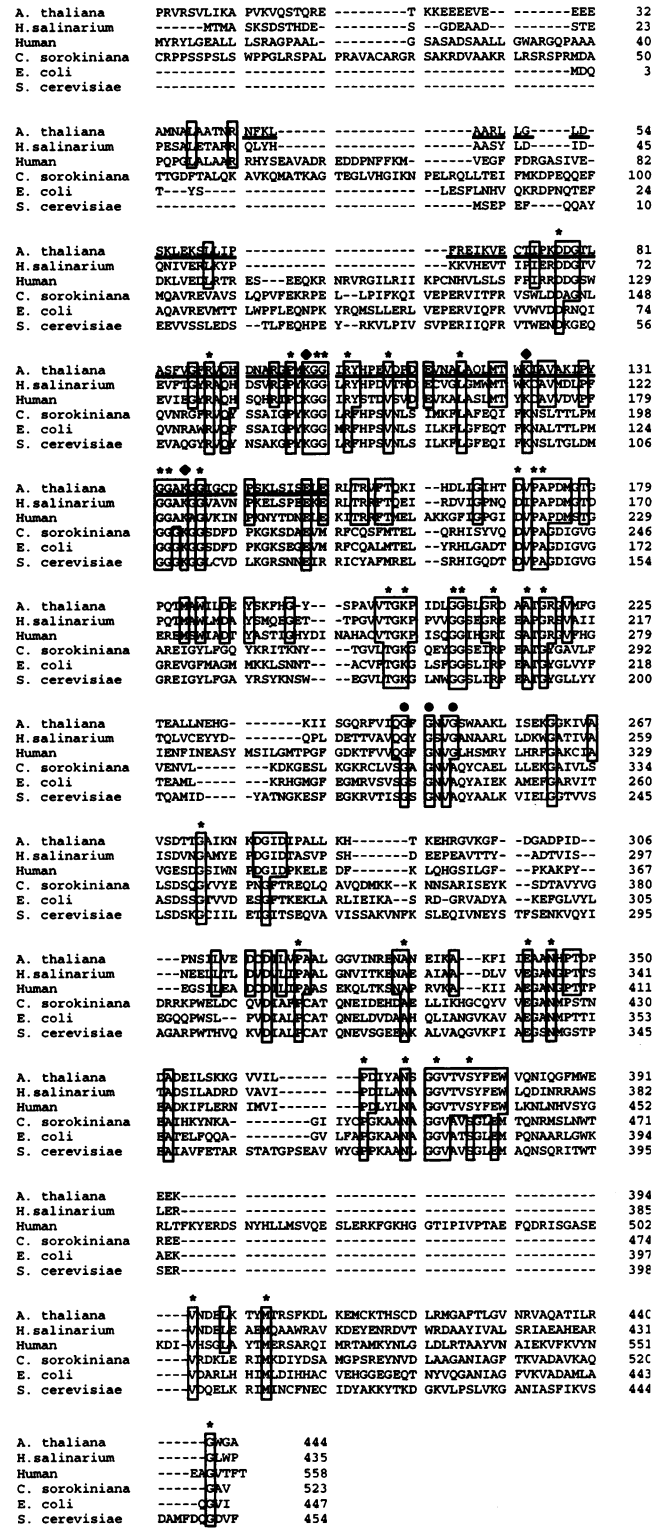

FIG. 1. Deduced protein sequence of *Arabidopsis* GDH1 and comparison with GDH sequences from other organisms. The deduced *Arabidopsis* GDH1 protein sequence was aligned to other known GDH proteins. Conserved amino acids are boxed. The asterisks denote residues conserved among all previously described GDH enzymes. Diamonds indicate residues involved in putative glutamate-binding site. Dots indicate residues involved in the formation of the putative NADH-binding site. Dashed lines represent sequence gaps that were created to allow the best alignment possible. Underlined residues denote the region encompassing the *GDH1* probe used in Northern and Southern analyses.

1, asterisks; ref. 37) including a putative glutamate-binding site (Fig. 1, diamonds) and a consensus sequence (GXGXXG) that forms a putative NADH-binding site (Fig. 1, dots; ref. 37). The translation product of *Arabidopsis GDH1* cDNA contains at its amino terminus features characteristic of mitochondrial targeting sequences as determined by the PSORT program (38). The first in-frame methionine is located at residue 34. As there is an open reading frame 5′ to this ATG, these additional sequences may also be part of a mitochondrial targeting sequence.

***Arabidopsis* Contains at Least Two Genes for GDH.** *GDH* sequences in *Arabidopsis* genomic DNA were identified by Southern blot analysis (Fig. 2). At low stringency, the *GDH1* cDNA probe detects at least two distinct genomic DNA fragments in each restriction digest (Fig. 2*A*). At high stringency, the *GDH1* probe detects a single DNA fragment in each lane (Fig. 2*B*). These results suggest the existence of at least two GDH genes in *Arabidopsis*, *GDH1* and *GDH2*.

***GDH1* Gene Regulation by Light.** Northern analysis was used to detect steady-state levels of *GDH1* mRNA using the *GDH1* probe at high stringency. The accumulation of *GDH1* mRNA was analyzed in various organs of mature *Arabidopsis* plants. In light-grown plants, *GDH1* mRNA accumulates to higher levels in leaves and flowers, compared with roots (Fig. 3*A*, lanes 2–4). Levels of *GDH1* mRNA present in leaves are induced in dark-adapted plants (Fig. 3*A*, lane 1). By contrast, levels of chloroplastic GS2 (*GLN2*) mRNA are reduced by dark adaptation (Fig. 3*A*, lane 1 compared with lane 2). Furthermore, when dark-adapted plants are re-exposed to light for increasing time intervals, a progressive repression of *GDH1* mRNA accumulation occurs (Fig. 3*B*). Conversely, GS2 mRNA accumulation was induced by the same light treatment (Fig. 3B). The reciprocal light regulation of mRNA levels for GDH1 and chloroplastic GS2 (*GLN2*) suggest that the encoded enzymes play distinct roles in plant nitrogen metabolism. Whether the other isoforms of these enzymes such as cytosolic GS1 or GDH2 play distinct or overlapping roles with chloroplastic GS2 and GDH1 remains to be determined.

The effects of light on *GDH1* mRNA accumulation in plants grown under a normal day/night cycle were also investigated (Fig. 3*C*). Low levels of *GDH1* mRNA accumulate at the end

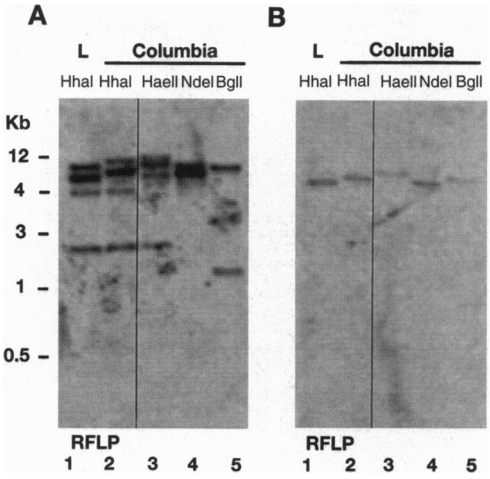

FIG. 2. Genomic Southern blot analysis of fragments encoding GDH in *Arabidopsis*. Genomic DNA of *Arabidopsis thaliana* was digested with the indicated restriction enzymes and analyzed by Southern blot. After hybridization the membranes were washed either at low stringency (1× SSC, 65°C, *A*) or at high stringency (0.1× SSC, 65°C, *B*). Lanes 1 and 2 show the restriction fragment length polymorphism produced after *Hha*I restriction digestion of genomic DNA from Landsberg (L) and Columbia ecotypes. The enzymes used in the Southern analysis do not contain an internal recognition site in the *GDH1* cDNA. The positions of the size marker bands are indicated on the left.

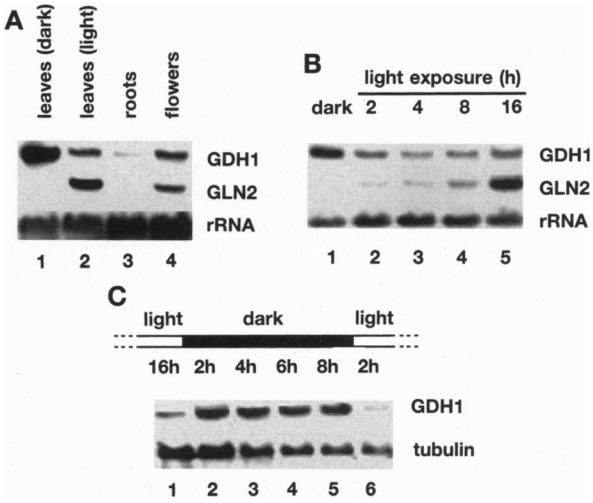

FIG. 3. Light inhibits accumulation of *GDH1* mRNA. (*A*) *A. thaliana* plants were grown in Vermiculite in a normal day/night cycle until bolting (6 weeks). Plants were then either transferred to continuous dark for 48 h (lane 1, leaves) or to continuous light for 48 h (lanes 2–4, leaves, roots, and flowers, respectively). (*B*) *A. thaliana* plants were grown semihydroponically in MS media supplemented with 3% sucrose for 16 days on a 16-h light/8-h dark cycle and then transferred to dark for 48 h (dark, lane 1). Thereafter, the plants were transferred to continuous light and samples were collected at 2, 4, 8, and 16 h (lanes 2–5). (*C*) *A. thaliana* plants were grown in soil for 4 weeks on a 16-h light/8-h dark cycle. Samples were collected every 2 h starting at the end of the light period (16 h light) (lane 1), throughout the dark period (lanes 2–5), and into the following light cycle (2 h light) (lane 6).

of the light period (Fig. 3*C*, lane 1). *GDH1* mRNA is induced 3-fold by 2 h of darkness, and remains elevated throughout the entire 8-h dark period (Fig. 3*C*, lanes 2–5). When plants are re-exposed to light for 2 h, *GDH1* mRNA returns to its initial low levels (compare lanes 1 and 6). α-Tubulin gene expression is not affected by the light/dark treatments. Thus, *GDH1* mRNA induction occurs during the dark phase of a normal day/night cycle and is repressed during the light cycle.

***GDH1* Gene Regulation by Carbon Metabolites.** The induction of *GDH1* mRNA by dark treatment can be a direct negative effect of light and/or an indirect "stress" effect caused by the depletion of carbon skeletons in dark-adapted plants (28). Light has been shown to exert direct effects on the expression of genes such as nitrate reductase or GS2 in several species (23, 39). Light can also exert indirect effects on these genes by modulating levels of carbon metabolites. Sucrose supplementation has been shown to induce the expression of genes for nitrate reductase and GS2 independent of light (40, 41). Previous biochemical studies have indicated reciprocal control of GS and GDH activities by light or sucrose (28, 42). In maize, it was shown that GDH activity, which is high in dark-stressed plants, is repressed when leaf discs are treated with sucrose (43). We tested whether the high levels of GDH1 mRNA induced by dark treatment in *Arabidopsis* could be repressed by sucrose (Fig. 4). The high levels of *GDH1* mRNA in plants dark-adapted for 48 h (Fig. 4, lane 1) are repressed by 3% sucrose (Fig. 4, lane 2). Conversely, chloroplastic GS2 (*GLN2*) mRNA accumulation is increased by sucrose supplementation (Fig. 4, lane 2). Repression of *GDH1* mRNA accumulation is not observed when a nonmetabolizable sugar (mannitol) is added to the medium (Fig. 4, lane 3). Thus, when carbon metabolites are high (*e.g.*, in light-grown plants or in plants grown in the presence of 3% sucrose), *GDH1* mRNA levels are low. Conversely, in carbon-starved plants (e.g., dark-adapted plants or plants grown on media with no exogenously supplied sucrose), *GDH1* mRNA levels are induced. These results suggest that metabolic regulation of GDH1

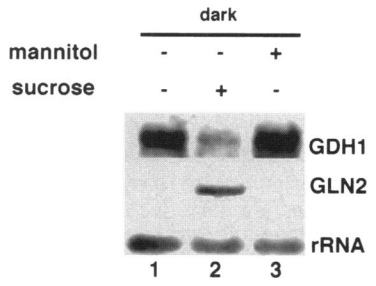

FIG. 4. Levels of carbon metabolites affect accumulation of *GDH1* mRNA. *A. thaliana* plants were grown semihydroponically in MS media (20 mM ammonia, 40 mM nitrate) supplemented with 3% sucrose for 16 days on a 16-h light/8-h dark cycle and then transferred to MS media containing no sucrose and incubated in the dark for 3 days. Thereafter, the plants were transferred to MS media either with no carbon source supplementation (lane 1), with 3% sucrose (lane 2), or with 3% mannitol (lane 3) and incubated in the dark for an additional 3 days.

expression may at least partially account for the induction observed by dark treatment.

Induction of *GDH1* mRNA by Exogenous Ammonia. Previous biochemical data demonstrated that GDH enzyme activity in *Arabidopsis* can be induced if plants are transferred to media containing 15 mM ammonia (28). This finding suggested a possible role for GDH in the assimilatory direction under conditions of ammonia excess. We assayed *GDH1* mRNA levels in plants grown on ammonia-free, nitrate-containing MS media supplemented with three different concentrations of ammonia (Fig. 5). In plants grown in a normal day/night cycle, levels of *GDH1* mRNA present in ammonia-free media (Fig. 5, lane 1) are induced 2- to 2.5-fold if the medium is supplemented with 20 or 40 mM ammonia, respectively (Fig. 5, lanes 2 and 3). In dark-adapted plants, the already high levels of *GDH1* mRNA cannot be further increased by ammonia supplementation (Fig. 5, lanes 4–6). In contrast, mRNA for chloroplastic GS2 (*GLN2*), which accumulates preferentially in light-grown plants, is unaffected by ammonia supplementation in either growth condition (Fig. 5).

Isolation and Genetic Characterization of *gdh1–1*, an *Arabidopsis* GDH-Deficient Mutant. *Arabidopsis* GDH has been previously shown to be a hexameric enzyme composed of two types of subunits (28, 29). Based on the genetic control of GDH isoenzyme variants, it was proposed that GDH is encoded by two genes in *Arabidopsis* (28, 29). Those conclusions, based on GDH isoenzyme studies, agree with our Southern blot (Fig. 2). To assess the relative *in vivo* functions

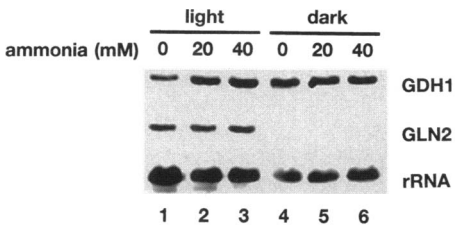

FIG. 5. *GDH1* mRNA accumulation is affected by exogenous ammonia. *A. thaliana* plants were grown for 16 days in MS media (20 mM ammonia, 40 mM nitrate) supplemented with 3% sucrose on a 16-h light/8-h dark cycle. Thereafter plants were transferred to MS media with no ammonia and incubated in either continuous light (light; lanes 1–3) or continuous dark (dark; lanes 4–6) for an additional 3 days. The plants were then transferred to ammonia-free MS media supplemented with 3% sucrose containing 19 mM nitrate in the presence of either 0 mM ammonia (lanes 1 and 4), 20 mM ammonia (lanes 2 and 5), or 40 mM ammonia (lanes 3 and 6) and left in continuous light (lanes 1–3) or continuous dark (lanes 4–6) for an additional 3 days.

of the *GDH1* and *GDH2* genes, we searched for *Arabidopsis* mutants deficient in either the GDH1 or GDH2 holoenzymes. Crude leaf protein extracts of ethyl methanesulfonate- and methylnitrosourea-mutagenized M_2 seedlings were assayed for GDH activity after nondenaturing gel electrophoresis. Leaf extracts of wild-type *Arabidopsis* contain seven GDH hexameric isoenzymes that are presumed to be the products of two GDH genes (Fig. 6, lanes 1 and 5).

In a screen of 8000 M_2 seedlings, we identified a single *Arabidopsis* mutant (*gdh1-1*) missing the GDH1 holoenzyme and the GDH1/GDH2 heterohexameric enzymes. The *gdh1-1* mutant possesses only the GDH2 homohexamer. When the *gdh1-1* mutant was allowed to self-pollinate, all M_3 progeny contained only the single GDH2 isoenzyme (Fig. 6, lanes 2–4), indicating that the original *gdh1-1* mutant was homozygous. All the F_1 progeny from a cross between the *gdh1-1* mutant and its wild-type parent contain the seven GDH isoenzymes of wild type, indicating that *gdh1-1* mutation is recessive (data not shown).

The *gdh1–1* Mutation Is Linked to the *GDH1* Structural Gene. Two types of genetic analysis were performed to determine whether the *gdh1-1* mutant is linked to the cloned *GDH1* gene. Homozygous mutants for the *gdh1-1* allele were identified in the F_2 progeny of a cross between the *gdh1-1* mutant (Columbia) and wild type (Landsberg) using the GDH isoenzyme gel assay. All mutant F_2 individuals (24 total) carried the *GDH1* gene of the mutant Columbia parent (data not shown). We further demonstrated that the *gdh1-1* mutation and the *GDH1* gene map to the same location on *Arabidopsis* chromosome 5. The *gdh1-1* mutation is located on chromosome 5, closely linked to the simple sequence length polymorphism marker nga106 (33.7 centimorgans) (3 recombination events in 24 individuals) (26). Separately, using the recombinant inbred lines (19), the *GDH1* gene was mapped to chromosome 5 at position 33.7 centimorgans, in the same vicinity of the *gdh1-1* mutation.

Phenotypic Characterization of the *gdh1–1* Mutation. Gene expression data revealed that *GDH1* mRNA levels increase in response to exogenously supplied inorganic nitrogen (Fig. 5) suggesting that the GDH1 gene product plays a role in ammonia assimilation under conditions of inorganic nitrogen excess. To test whether GDH1 actually plays such a role *in vivo*, we examined whether the *gdh1-1* mutant was specifically impaired in growth when grown on increasing concentrations of inorganic nitrogen. M_3 seeds of the *gdh1-1* mutant and an isogenic wild-type *Arabidopsis* line (Columbia) were sown side-by-side on agar plates oriented vertically so that root length could be used as a measure of seedling growth rate (Fig. 7). Plants were grown in a normal day/night cycle on ammonia-free/nitrate-free MS media supplemented with different concentrations of ammonia/nitrate with 3% sucrose and vitamins (Fig. 7 *A–C*) or without supplementation of vitamins (Fig. 7*D*). There is no difference in growth between *gdh1-1* and wild type when plants are

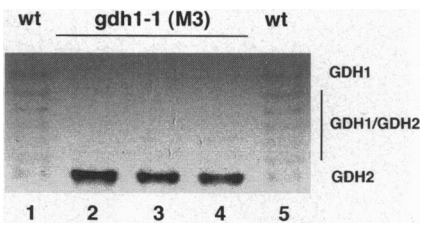

FIG. 6. GDH activity in wild-type *Arabidopsis* and *gdh1-1* mutant. Crude leaf protein extracts were made from rosette leaves of 21-day-old *Arabidopsis* plants, separated by electrophoresis on a nondenaturing polyacrylamide gel, and stained for GDH activity. Lanes 1 and 5, extract of wild-type *Arabidopsis* (Columbia). The seven holoenzymes result from the formation of two homohexamers (GDH1 and GDH2), and five heterohexamers of GDH are indicated on the right (GDH1/GDH2). M_3 individuals from a selfed *gdh1-1* mutant display only the GDH2 homohexamer (lanes 2–4).

grown on inorganic nitrogen-free MS media (Fig. 7*A*). *Arabidopsis* wild-type plants grown under intermediate levels of inorganic nitrogen (Fig. 7*B*) display a better overall growth phenotype compared with plants grown on high inorganic nitrogen (Fig. 7*C*). The *gdh1-1* mutant displays an impaired root growth phenotype and mild shoot chlorosis compared with wild type when grown under either intermediate or high inorganic nitrogen conditions (Fig. 7 *B* and *C*). This growth defect and chlorosis of the *gdh1-1* mutant is exaggerated when plants are grown in high concentrations of inorganic nitrogen under suboptimal conditions (e.g., without vitamins) (Fig. 7*D*). The fact that the *gdh1-1* mutant displays a growth defect specifically in the presence of exogenously supplied inorganic nitrogen supports the notion that GDH1 plays a role distinct from that of GS in ammonia assimilation.

DISCUSSION

The analysis of *GDH* gene regulation and preliminary characterization of a *gdh*-deficient *Arabidopsis* mutant (*gdh1-1*) detailed herein suggest that GDH plays a unique role in plant nitrogen assimilation. Our findings that the expression of the *Arabidopsis GDH1* gene is regulated by light and/or metabolites is consistent with an *in vivo* role for GDH in regulating a balance between carbon and nitrogen metabolites. Earlier biochemical studies showed that GDH activity increases in carbon-starved plant cells (4, 7). Here we demonstrate that *GDH1* mRNA accumulates specifically in dark-adapted (or carbon-starved) plants. These induced levels of *GDH1* mRNA are specifically repressed by light or by the addition of an exogenous carbon source to the growth media such as sucrose. Moreover, the light effect on the *GDH1* mRNA levels was also observed in plants grown under a normal day/night cycle. These findings suggest that under conditions of low carbon availability (in the dark-adapted or the carbon-starved plants), induced levels of GDH1 function to catabolize glutamate to provide 2-oxoglutarate for the tricarboxylic acid cycle.

Our data on *GDH1* gene regulation also support the notion that GDH1 plays a role in the direction of nitrogen assimilation under certain growth conditions. Low levels of *GDH1* mRNA present in light-grown plants are induced by the addition of ammonia to the growth media. These molecular data suggest that GDH1 may play an accessory role to GS/GOGAT in primary nitrogen assimilation in plants grown in the presence of inorganic nitrogen. Moreover, the fact that no GS mutants were uncovered in an *Arabidopsis* photorespiratory mutant screen, combined with the fact that the *Arabidopsis* ferredoxin-GOGAT-deficient mutant phenotype (13) is only mildly chlorotic suggests that another enzyme(s) may be capable of assimilating some amount of photorespiratory ammonia in *Arabidopsis*. GDH is a possible candidate for such a role in C_3 plants.

Using a GDH enzyme assay screen, we identified an *Arabidopsis* mutant (*gdh1-1*) defective in one of two GDH gene products. Whereas in wild-type *Arabidopsis*, the GDH1 and GDH2 gene products form homohexamers and heterohexamers, only the GDH2 homohexamer is detected in the *gdh1-1* mutant. We have shown that this enzyme defect in the *gdh1-1* mutant is genetically linked to the *GDH1* structural gene. There are several types of mutations in the *GDH1* gene which could lead to the absence of the GDH1 homohexamer and the loss of the GDH1/GDH2 heterohexamers. (*i*) A mutant GDH1 subunit could act in a dominant-negative fashion to assemble with and inactivate GDH1 homo- or heterohexameric holoenzymes. We have ruled out this possibility, as the *gdh1-1* mutation behaves in a recessive fashion. (*ii*) A mutant GDH1 subunit could be synthesized, but be unable to assemble into a homohexamer or into heterohexamers. (*iii*) The GDH1 subunit may not be synthesized or may be unstable in the *gdh1-1* mutant. At present, we cannot distinguish between these last two possibilities. In either case, the *gdh1-1* mutant that contains no detectable isoenzymes for GDH1 or the GDH1/GDH2 holoenzymes displays a conditional growth phenotype.

A single *gdh1-1* allele was identified in our screen of 8000 M_2 seedlings, and 14 independent mutants deficient in one of two aspartate aminotransferase isoenzymes were identified in the same screen (C. Schultz and G.M.C., unpublished data). There are several possible explanations for the paucity of *gdh*-deficient mutants identified in this screen. First, it is possible that the majority of mutations in *GDH1* are lethal and that the single viable *gdh1-1* mutant recovered is leaky. An alternative explanation is that most mutations in the *GDH1* gene are leaky and do not result in the complete loss of the GDH1 holoenzyme. In this case, the *gdh1-1* mutant we recovered represents a strong, possibly null allele. Finally, it is possible that the mutation in *gdh1-1* is a rare mutation that affects the residue(s) involved in the assembly of the homohexamer and heterohexamers. Future characterization of the molecular lesion in *gdh1-1* should allow us to discriminate the nature of the mutation in the *GDH1* gene.

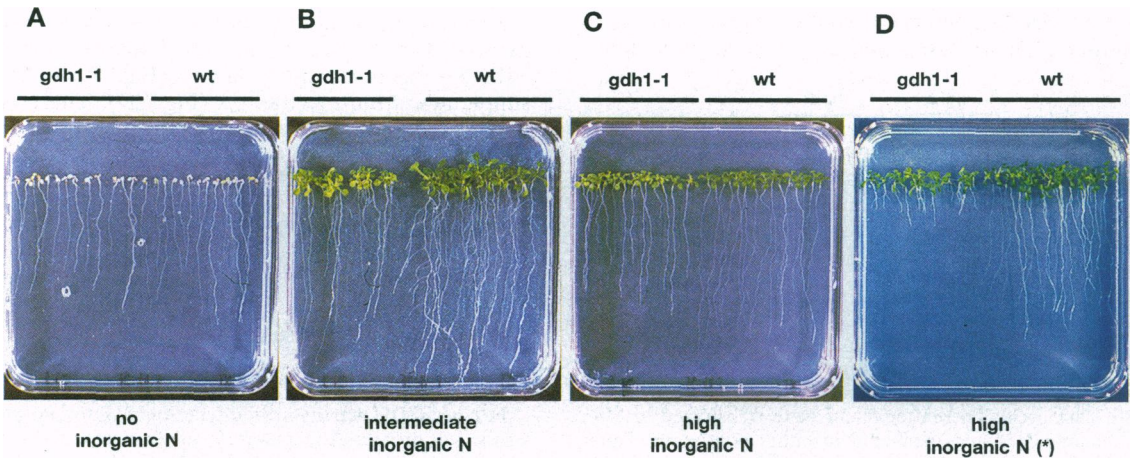

FIG. 7. Growth phenotype of the *gdh1-1* mutant. Growth of wild-type *Arabidopsis* versus the *gdh1-1* mutant seedlings was measured in a vertical root length assay. Wild-type (wt) and M_3 seeds of the *gdh1-1* mutant were sown side-by-side on ammonia-free/nitrate-free MS media containing vitamins and 3% sucrose supplemented with either no inorganic nitrogen (0 mM ammonia, 0 mM nitrate; *A*), intermediate levels of inorganic nitrogen (2 mM ammonia, 4 mM nitrate; *B*), or high levels of inorganic nitrogen (20 mM ammonia, 40 mM nitrate; *C*). (*D*) Plants grown on MS media supplemented with 3% sucrose containing high levels of inorganic nitrogen (20 mM ammonia, 40 mM nitrate) without the vitamin supplement (*). Plates were incubated vertically for 12 days and grown under a normal day/night cycle.

The *Arabidopsis gdh1-1* mutant is analogous to the previously isolated maize GDH-deficient mutant (18) in that they are both deficient in the GDH1 homohexamer and in heterohexamer production. Mutants lacking the GDH2 homohexamer and the GDH1/GDH2 heterohexamers were not recovered in our screen nor were such mutants identified in maize (18), raising the possibility that a mutation in the *GDH2* gene is lethal. The previously identified "*GDH1*-null" maize mutant retains 10% of the wild-type GDH activity (17, 18). It was reported that seedlings homozygous for *GDH1*-null are phenotypically indistinguishable from wild-type siblings unless grown under low night temperatures (17). In a subsequent study, the *GDH1*-null mutant was compared with a related, but not strictly isogenic, strain that is wild-type for GDH (18). In that study, the *GDH1*-null mutant was reported to have a lower shoot/root ratio and a 40–50% lower rate of assimilation of $[^{15}N]NH_4$. These results, although suggestive of an anabolic role for maize GDH1, are not conclusive, as the maize *gdh* mutant strain and wild-type strain used were not isogenic. Furthermore, as maize is a C_4 plant, the maize *GDH1*-null mutants cannot be used to assess the importance of GDH in the assimilation of photorespiratory ammonia.

A preliminary growth analysis on the M_3 generation of the *gdh1-1 Arabidopsis* mutant was performed to assess the *in vivo* function of the GDH1 gene product in a C_3 plant. The *gdh1-1* mutant displays a retarded growth phenotype compared to wild type, which is conditional on the addition of inorganic nitrogen to the growth media. The *gdh1-1* plants show a growth rate reduction and mild shoot chlorosis when plants are grown on media containing intermediate and high inorganic nitrogen levels. This growth defect is exaggerated when plants are grown on high inorganic nitrogen under suboptimal conditions (*e.g.*, without vitamins), which suggests that GDH1 plays an especially important role in nitrogen assimilation under conditions of plant stress. Previous biochemical data support the notion that GDH enzyme plays an important role in plants grown under stress conditions (6).

It should be noted that neither the *gdh* mutant in maize nor the *Arabidopsis gdh1-1* mutant described here is null for GDH activity, as a second gene for GDH2 is unaffected. A new mutant screen has been initiated to search for additional alleles of *gdh1* in *Arabidopsis* and for putative mutants in the *GDH2* gene. An allelic series of mutants in either *GDH* gene as well as the creation of double mutants will be useful to assess all the *in vivo* roles of GDH in nitrogen use in plants.

Our gene expression data suggest that GDH1 plays an unique role in nitrogen assimilation compared with GS. This notion is supported by the gdh1-1 mutant phenotype and by the fact that light and sucrose each induce the accumulation of mRNA for chloroplastic GS2 yet repress the accumulation of *GDH1* mRNA. The reciprocal regulation of GDH1 and chloroplastic GS2 by light or sucrose at the gene expression level mirrors that observed at the level of enzyme activity (43). It will be interesting to determine whether the reciprocal regulation of the GDH1 and chloroplastic GS2 genes is mediated via a common mechanism.

Note Added in Proof. A report on maize *GDH* gene by Sakakibara *et al.* (44) was published while this paper was in press.

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